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by Tomasz Krupnik

Abstract:

Sustainability of life depends on the ability of living organisms to respond to external stimuli. This function is facilitated by transmembrane proteins, capable of transducing information about the environment and transporting solutes from the environment through the membrane to the inside of the cell. The glutamate symporter GltS of Escherichia coli and the citrate symporter CitS of Klebsiella pneumonia are examples of transport proteins. These two proteins share little or no sequence similarity, yet the hydropathy profiles of the sequences are remarkably similar. For that reason they are thought to have the same general fold of the polypeptide chain and mechanism of transport. In both of them it is possible to distinguish two domains consisting of five transmembrane elements, a reentrant or pore loop and a long cytoplasmic loop. Moreover, both are dimers. The dimeric quaternary structure most likely forms a basin at the interface of the monomers, a feature observable by electron microscopy analysis of CitS. Crosslinking experiments performed on purifies sample of GltS and CitS with the unspecific crosslinker glutaraldehyde revealed only CitS as dimer. Crosslinking of GltS was observed only after fusing an additional protein mass to the transporter. Similar fusion experiments allowed the conclusion that the large cytoplasmic loops are placed along the long axis, away from the dimer interface. By studying the accessibility of two introduced cysteine residues it was demonstrated that changes in the large cytoplasmic loop are transduced to the active center, opening up the possibility of regulation of activity through the loop.